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Investigation of Association Between *PstI* Polymorphism of Tyrosine Hydroxylase (TH) Gene and Autism Using PCR-RFLP Technique

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Abstract: Autism is a syndrome with multiple nongenetic and genetic causes. Individuals with autism display biochemical abnormalities in monoamines. Tyrosine hydroxylase (TH) gene encodes rate limiting enzyme in the synthesis of dopamine. Changes in TH gene expression or function may influence the processes or behaviors modulated by dopamine. The aim of the present study is to investigate the association of *Pst I* polymorphism at TH gene and autism in Saudis autistic children. PCR-RFLP technique was used to genotype rs2070762T/C polymorphism with *Pst I* of Tyrosine hydroxylase gene among 50 autistic patients and 50 typically healthy individual children. Statistically no significant association was detected between studied polymorphism and autism (overall $X^2 = 3.79, P = 0.15$).

Key words: Polymorphism · Tyrosine hydroxylase gene · Autism · PCR-RFLP

INTRODUCTION

Autism is a pervasive neurodevelopmental disorder that impairs the normal development of social and emotional interactions and related forms of communication. Its symptoms are characterized by a triad consisting of limited or absent verbal and nonverbal communication, a lack of reciprocal social interaction or responsiveness and restricted, stereotypical and ritualized patterns of interests and behavior [1, 2]. Autism is the most severe manifestation of a broad spectrum of disorders, known as autistic spectrum disorders (ASD) that share these essential features, but vary in their degree of severity and/or age of onset. The world-wide prevalence of ASD is 58.7 per 10,000 individuals [3] with male: female ratio of 4:1. Genetic involvement of the disorder has been manifested through twin and family-based studies [4] and these familial connections have paved the path for numerous genome-wide scans to identify the autism susceptibility regions in the human genome. A strong genetic basis is accepted [4-6] and the estimated heritability of autism is upwards of 90%, which is higher than many other complex genetic disorders such as breast cancer [7] or type 1 diabetes30. However, the etiology of autism is poorly understood both at the cellular and the molecular level. The tyrosine hydroxylase gene consists of fourteen exons [8] and maps to chromosome 11p15 [9]. Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of dopamine and norepinephrine [10]. Dopamine modulates a broad variety of processes, functions and behaviors that are abnormal in individuals with ASDs including motor functions, cognitive processes, emotional regulation, social interaction and homeostatic processes such as blood pressure, sleep patterns and GI function. Changes in TH gene expression or function may influence such processes or behaviors that are modulated by dopamine. Different association studies with different experimental designs have been conducted to examine the role of *TH* gene as a candidate gene in the etiology of autism using various PCR based techniques [11-15]. The fluctuated results from previous studies were due to differences in measured genetic end points, used experimental design and techniques. The present study aims to investigate the association between rs2070762 allelic fragments among TH gene and autism amongst Saudis population using PCR-RFLP technique with *Pst I* restriction enzyme.

MATERIALS AND METHODS

Subjects: The sample of study consisted of 100 unrelated subjects. Fifty autistic patients were selected. Fifty age matched clinically normal children from the same geographic and socioeconomic group were selected as

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control group. Children with any neurological condition suspected to be associated with autism were excluded from control group. The diagnosis of autism was confirmed for all cases using the Autism Diagnostic Interview-Revised [16] and the Autism Diagnostic Observation Schedule, modules 1, 2 or 3 [17-19]. Oral informed consent was given to all individuals for participation in this study and all institutional requirements were met.

Blood Samples Collection and PCR-RFLP Assay: Blood samples were collected in anticoagulants EDTA tubes. The polymorphism, rs2070762 T/C, was genotyped at the TH locus. PCR amplification of The polymorphisms, rs2070762 T/C, was carried out with annealing temperature 54°C using Phusion Blood Direct PCR kit (Finnzymes) with forward primer 5'- CAGCCCTACCAAGACCAGAC-3' and the reverse primer 5'- GTCCTTCTCACGGATGGTGT-3' [15]. PCR product was eletrophoresed on 1.5% agarose (Bioshop Canada) with DNA ladder standard 100 bp (Bioshop Canada). All gels were stained with 0.5 μg/ml ethidum bromide. The rs2070762 amplified 270 bp fragment was visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene, Frederick, Maryland, USA). The digital image files were analyzed using Gene Tools software from Syngene. The *Pst I* restriction enzyme was used to cleave the rs2070762 amplified 270 bp fragment into cut (C allele) or uncut (T allele). The cleaved bands were visualized after electrophoresis on 2.5% agarose gel with DNA ladder standard 50 bp (Bioshop Canada).

Statistical Analysis: Allele frequencies of the polymorphism, rs2070762 T/C were calculated by single gene counting method. Chi-square test was done using SPSS Package to compare the genotype frequencies between cases and controls. Association between studied SNP and Autism was expressed as odds ratio (OR) and its 95% confidence interval (95% CI) was calculated. Statistical packages used for the analysis were Epi-Info-version 6 and SPSS version 16.

RESULTS

According to the electrophoretic banding pattern of cleaved bands showed in fig. 1 and 2, three different genotypes were detected. Table 1 shows the distribution of genotype frequencies of rs2070762 polymorphism between cases and controls. In TT genotype a single band of length 270 bp was detected; in TC genotype, bands with length 270, 230 and 40 were observed and in CC genotype, bands of 230 and 40 were obtained.

Among 50 autistic patients 12 (24%) were TT genotype, 14 (28%) were TC genotype and 24 (48%) were CC genotype. On the other hand among 50 control individuals 19 (38%) were TT genotype, 16 (32%) were TC genotype and 15 (30%) were CC genotype. Allele frequency of the rs2070762 T/C polymorphism was calculated and compared amongst patients and controls groups. The C and T allele frequencies were 0.62 and 0.38 in cases and controls respectively. On the other hand these frequencies were 0.46 and 0.54 amongst cases and controls respectively. Chi-square test was used through SPSS Package to compare the genotype frequencies between cases and controls. Statistically no significant differences between frequency of studied genotypes amongst cases and controls were observed (overall $X^2 = 3.79$, $P = 0.15$) Tab. 2.

Table 1: Distribution of genotype frequencies of rs2070762 polymorphism between cases and controls

	Genotypes							
	TT (270 bp)		TC (270, 230, 40 bp)		CC (230, 40 bp)			
	n	%	*N*	%	*n*	%	F(C)	F(T)
Cases, n=50	12	24%	14	28%	24	48%	0.62	0.38
Controls, n=50	19	38%	16	32%	15	30%	0.46	0.54

Table 2: Genotypes and allele frequencies of rs2070762 T/C polymorphism

Genotypes	Cases (n=50)	Control (n=50)	OR (95% CI)	Significant
TT	12 (24%)	19 (38%)	0.52 (0.2, 1.33)	NS
TC	14 (28%)	16 (32%)	0.83 (0.32, 2.12)	NS
CC	24 (48%)	15 (30%)	2.15 (0.88, 5.33)	NS

Chi square= 3.79 df=2 p value= 0.15 (Not significant)

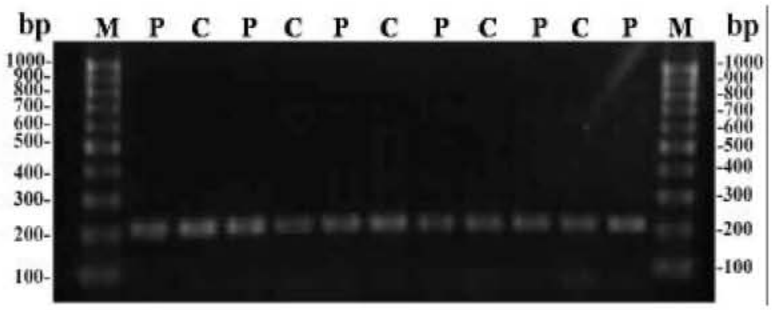

Fig. 1: PCR amplification of rs2070762 polymorphism at *TH* locus among autistic patients (P) and healthy control subjects (C)

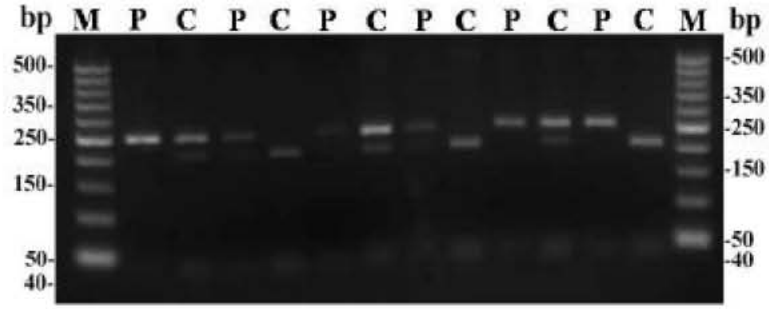

Fig. 1: PCR-RFLP analysis of rs2070762 polymorphism at *TH* locus with Pst I among autistic patients (P) and healthy control subjects (C)

DISCUSSION

Autism is a neurodevelopmental disability characterized by deficits in verbal communications, impairments in social interactions and repetitive behaviors. Brain pathology is extensive, suggesting widespread dysfunction of neurotransmitter systems. Genetic, biochemical and gene association studies have shown that a number of neurotransmitters including serotonin, dopamine, oxytocin, GABA and glutamate and acetylcholine contribute to the pathology of autism [20]. Several studies have indicated strong involvement of multigenic components in the etiology of autism [21].

Tyrosine Hydroxylase converts phenylalanine to dopamine and is rate limiting enzyme involved in the synthesis of catecholamines [22]. Therefore it is of significant interest as a candidate gene in studies of Autism.

In the present study, the association of polymorphism rs2070762 T/C at the TH locus with autism was investigated by PCR-RFLP technique with Pst I restriction enzyme in 50 autistic children and 50 healthy matched subjects. The obtained results confirmed that, there is no significant association between polymorphism rs2070762 T/C at the TH locus with autism. This result was consistent with previous study [14, 23]. On contrast, association between TH gene polymorphisms and risk from mothers having sons with ADS has been reported [15]. There are several possible explanations for the differences in the obtained results between the present study and the Hettinger study [15]. Family based linkage and association studies were carried out within Hettinger study [15] while case control study was implemented in the present study. Also sample size was different in the two studies. In addition negative results may be due to heterogeneity among Saudis autistic studied patients. Several evidences demonstrated that no association between Tyrosine Hydroxylase gen polymorphisms with autism, Tourette syndrome, or ADHD [24], Suicide Victims [25], affective disorder [26], Schizophrenia [27,28], personality traits in healthy Japanese subjects [29].

Finally larger sample and combination of more than one polymorphism at TH locus may be needed to detect a small effect of susceptible polymorphisms.

CONCLUSION

The present study suggested that no significant association was detected between rs2070762 polymorphism at the *TH* locus and autism. Replication of this study in large independent samples is also essential to help clarify whether rs2070762 polymorphism at the *TH*

locus related to Autism susceptibility. Further studies focused on combination studies between more than one *TH* polymorphism as well as interaction of the loci between *TH* and other genes and their potential roles in the pathophysiology of autism were needed.

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